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PENSOFT

First record of a potentially introduced leech, *Helobdella* aff. *robusta* Shankland, Bissen & Weisblat, 1992 (Hirudinea, Glossiphoniidae), in Japan

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Abstract. *Helobdella* aff. *robusta* Shankland, Bissen & Weisblat, 1992 is recorded for the first time from Japan. The haplotype of the cytochrome *c* oxidase subunit I (COI) gene sequence of a specimen from Japan is identical to that of one collected in California, USA. The dorsal pigmentation pattern of the Japanese specimens is different from that of *H. robusta* inhabiting North America, but nonetheless, most of their morphological characteristics are consistent with the diagnostic features of this species. This species is considered to be of New World origin, and the Japanese population was likely established from a recent introduction.

Keywords. COI, DNA barcoding, introduced species

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Introduction

The freshwater leech genus *Helobdella* Blanchard, 1896 is recognized as the most diverse group in the family Glossiphoniidae with about 80 valid species (Christoffersen 2009). The vast majority of the described species of this genus are distributed in South America, which suggests that *Helobdella* originated in this region (Siddall et al. 2005). *Helobdella* species have expanded into various regions and are now found on all continents except Antarctica; however, several species are known to be introduced species (Oceguera-Figueroa et al. 2010).

Helobdella europaea Kutschera, 1987 is the most widely introduced species that is probably native to North America (Morhun et al. 2021), but it has also been recorded in Europe, Oceania, Africa, and Taiwan (Kutschera 1985; Siddall and Budinoff 2005; Lai et al. 2009; Reyes-Prieto et al. 2014). Another obviously introduced species is *H. octatestisaca* Lai & Chang, 2009. This species is known to occur in Taiwan, Mexico, South Africa, Unites States, and Spain and appears to have been introduced from central Mexico (Lai et al. 2009; Oceguera-Figueroa et al. 2010; Richardson et al. 2017; Perera et al. 2019).

In Japan, only one species of this genus is known to date, which appears to belong to the *H. stagnalis* (Linnaeus, 1758) species complex due to the presence of a small chitinous scute on the dorsal side of the neck (e.g., Oka 1935; Itoh 2001; Iwama et al. 2019). However, in this study, *Helobdella* specimens without a nuchal scute were collected from Japan. Based on mitochondrial DNA sequences, the specimens were identified as *Helobdella* aff. *robusta* Shankland, Bissen & Weisblat, 1992, which is known to occur in the United States and Mexico (Fig. 1; Shankland et al. 1992; Oceguera-Figueroa et al. 2010). These specimens represent the first occurrence of this species outside of the Americas.

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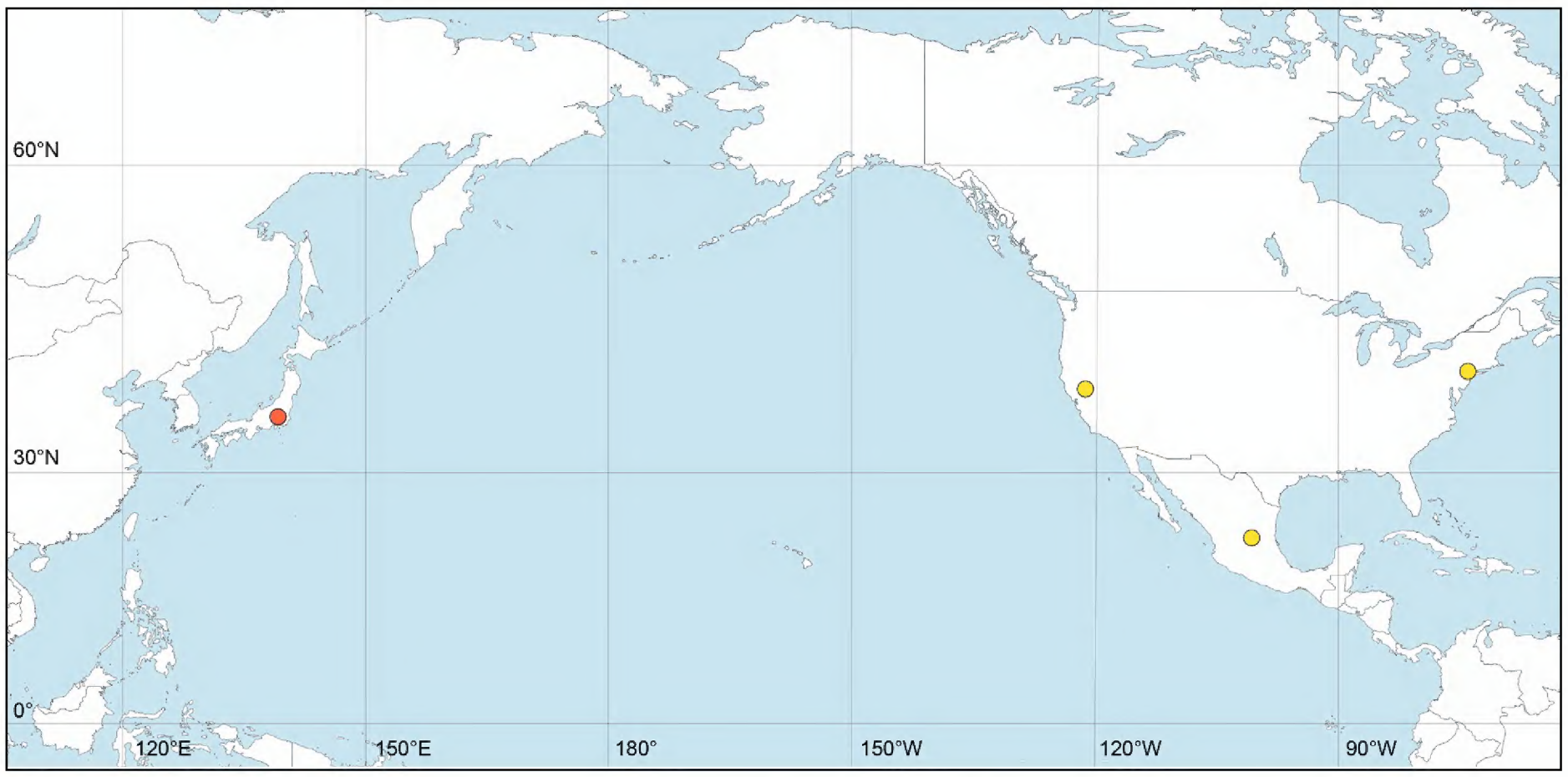

Figure 1. Map showing the collection localities. The red circle denotes the new locality, and the yellow circles are from Bely and Weisblat (2006) and Oceguera-Figueroa et al. (2010). This map was created with mapchart.net.

Methods

Leech specimens were collected from beneath a stone in a small stream flowing into the Sakai River, Kanagawa, Japan. The specimens were relaxed by the gradual addition of 99% ethanol to freshwater and fixed in 70% ethanol. Three specimens were compressed between two glass slides, stained with a mixture of Mayer's paracarmine and Ehrlich's hematoxylin, and then mounted on slides. Four measurements were taken on uncompressed specimens: body length from the anterior margin of the oral sucker to the posterior margin of the caudal sucker (BL), maximum body width (BW), caudal sucker length from the anterior to the posterior margin of the sucker (CL), and caudal sucker width from the right to the left margin of the sucker (CW). These specimens were deposited in the Zoological Collection of Kyoto University (KUZ).

Total DNA of a representative specimen (KUZ Z4515) was extracted from the part of the caudal sucker using phenol/chloroform extraction. The partial sequence of the cytochrome *c* oxidase subunit I (COI) gene was PCR amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR and cycle sequencing reactions were performed according to Nakano (2012), except for the use of PCR reaction kit, EmeraldAmp PCR Master Mix (Takara Bio). The newly determined COI sequence was deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (DDBJ). *Helobdella* leeches that lack a nuchal scute and bear longitudinal stripes on the dorsal surface, as observed in the present specimens, have been known to form a monophyletic group (Oceguera-Figueroa et al. 2010). Accordingly, the newly determined COI sequence (658 bp; INSD accession number LC761207) was aligned with eight available sequences of *H.* aff. *robusta*, as well as four sequences of scuteless congeners, which were obtained from INSD, using MAFFT v. 7.427 (Katoh and Standley 2013). All of the four sequences were determined based on the scuteless specimens identified by Siddall and Borda (2003) and Kutschera et al. (2013). Phylogenetic analysis was conducted using IQ-TREE v. 1.6.12 (Nguyen et al. 2015) with the best-fit model of HKY + F + G4 selected automatically and 1000 nonparametric bootstrapping replicates.

Results

Family Glossiphoniidae Vaillant, 1890
Genus *Helobdella* Blanchard, 1896

Helobdella aff. *robusta* Shankland, Bissen & Weisblat, 1992

Figure 2

New record. JAPAN – **Kanagawa** • Yokohama, Izumi, Simoiida-cho, Sakai River; 35°23′53″N, 139°28′43″E; 13.IX.2022; N. Kambayashi leg.; 1 specimen, KUZ Z4515; DDBJ LC761207; 08.X.2022; C. Kambayashi leg.; 16 specimens, KUZ Z4516–Z4519.

Identification. Body lanceolate, broadest in posterior half (Fig. 2). Measurements (mean, followed by ranges in parentheses; n = 14, KUZ Z4515–Z4516): BL 6.04 mm (3.70–7.89), BW 2.34 mm (1.45–3.42), CL 1.04 mm (0.67–1.54), CW 0.97 mm (0.60–1.50). Somites I–II fused. Somite III uniannulate. Somite IV biannulate, (a1 + a2) = a3. Somites V biannulate, (a1 + a2) > a3. Somites VI–XXIV triannulate, a1 = a2 = a3. Somites XXV and XXVI biannulate, (a1 + a2) > a3. Somite XXVII uniannulate. Anus on somite XXVII. Male gonopore in somite XII a1/a2. Female gonopore in somite XII a2/a3. Gonopores separated by 1 annulus. Eyespots in 1 pair on anterior margin of somite III;

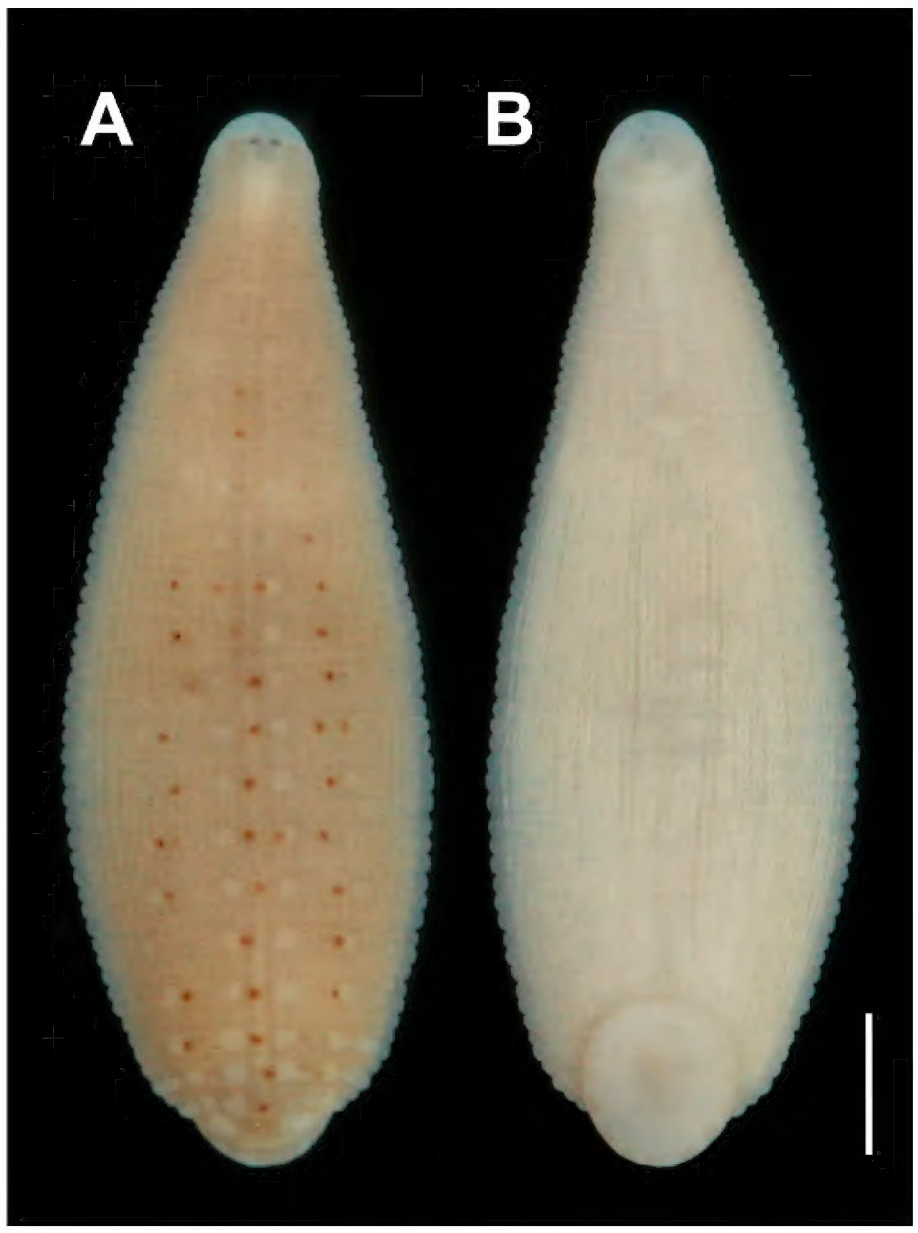

Figure 2. *Helobdella* aff. *robusta* from Japan, KUZ Z4516. **A.** Dorsal view. **B.** Ventral view. Scale bar = 1 mm.

punctiform and well separated between them. Salivary glands diffuse. Crop caeca in 5 pairs; 1st–4th pairs crop caeca simple, nondiverticulated; 5th pair deflected posteriorly, extend to about XXIII obliquely forming postcaeca or diverticula.

In life, dorsal surface generally orangish brown with faint longitudinal stripes; color faded in preservative. Dorsal surface bearing up to 3 longitudinal rows of darkly pigmented papillae situated on middle annuli of each of somites X–XXVII. Cream-colored pigment spots located immediately lateral to rows of black papillae. Venter flat without papillae or stripes. No mid-dorsal scute in head region of body.

The obtained COI sequence was identical with one of the haplotypes of *Helobdella* aff. *robusta* collected in California, USA (INSD accession number DQ995302). Molecular phylogenetic analysis revealed that Japanese population of this species belongs to the clade of "*Helobdella* sp. 1" designated by Bely and Weisblat (2006), and is most closely related to *H. lineata* (Verrill, 1874) (Fig. 3).

Discussion

Based on the COI sequence analysis, the *Helobdella* specimens collected in this study are identified as *H.* aff. *robusta*. Although most of the morphological characteristics were consistent with the diagnostic features of this species, there were some disparities in pigmentation patterns, such as the presence of five rows of black papillae in the original description (Shankland et al. 1992) compared with the presence of three rows in this study. Nevertheless, these variations are recognized in the congeneric species *H. austinensis* Kutschera, Langguth, Kuo, Weisblat & Shankland, 2013 (Kutschera et al. 2013). Consequently, the pigmentation pattern observed in the present study can be considered a variant of *H.* aff. *robusta*.

Helobdella robusta was originally described based on specimens collected from a creek in California,

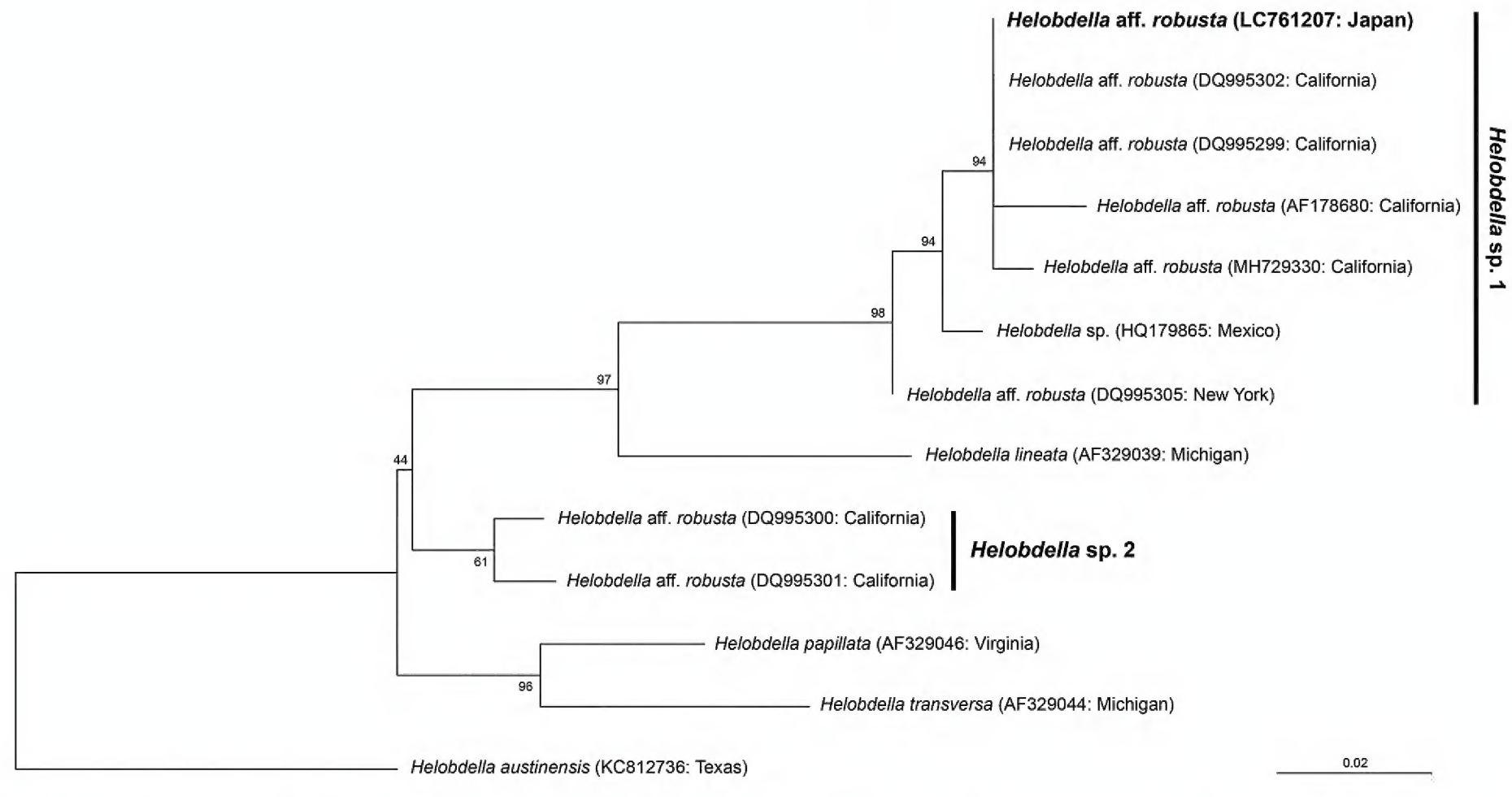

Figure 3. Maximum-likelihood tree of COI sequences. INSD accession numbers and localities are presented in parentheses. Numbers on nodes indicate bootstrap values. *Helobdella* sp. 1 and *Helobdella* sp. 2 were designated based on the descriptions in Bely and Weisblat (2006).

USA. Subsequent genetic analyses revealed the existence of two distinct lineages within the type locality, which were designated as "*Helobdella* sp. 1" and "*Helobdella* sp. 2" (Bely and Weisblat 2006). The former species, which includes the specimens obtained in this study, has been recorded from California and New York in the United States and San Luis Potosí in Mexico (Bely and Weisblat 2006; Oceguera-Figueroa et al. 2010). The close relatives of this species are concentrated in North and Central America (Iwama et al. 2019), which suggests that "*Helobdella* sp. 1" originated in this geographic region. Because *Helobdella* species without a nuchal scute have never been reported in Japan despite consistent surveys until the 1930s (Oka 1935), the "*Helobdella* sp. 1" population obtained in this study was most likely recently introduced from the Americas.

Leech accidental introduction has been proposed to be associated with the international trade of exotic organisms (Bennike 1943; Reyes-Prieto et al. 2014). In particular, some species of *Helobdella* leeches are known to reproduce through self-fertilization (Iyer et al. 2019), which may contribute to their ability to efficiently establish populations in newly introduced environments. Future studies are required to assess the impact of *Helobdella* invasions on native freshwater ecosystems.

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Author Contributions

Conceptualization: CK. Data curation: CK, TN. Visualization: CK. Writing – original draft: CK. Supervision: TN. Validation: TN. Writing – review and editing: TN.

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